PHAGE-DEPENDENT SUPERPRODUCTION OF BIOLOGICALLY ACTIVE PROTEIN AND PEPTIDES

Related Application

The present application claims priority to provisional patent application serial no. 60/225,437, entitled, "Phage-Dependent Superproduction of Biologically Active Protein and Peptides," filed on August 15, 2000.

Background of the Invention

Field of the Invention

This invention relates to recombinant DNA technology and more particularly to a new method for enhancing the production of heterologous proteins in bacterial host cells. The disclosed method involves infecting host cells, which contain plasmid encoding the gene of interest operably linked to the T7 promoter, with bacteriophage λ to induce lysis of the bacterial host cells. Super-production may be achieved in selected host cells either when the plasmid alone carries at least one copy of the heterologous DNA or when both plasmid and phage λ each carry at least one copy of the heterologous DNA.

Description of the Related Art

At present, genetic engineering methods allow creating microorganism strains capable of producing substantial amounts of various bioactive substances having important applications in medicine and industry. Typically, plasmid vectors into which a heterologous gene has been inserted are used to transform bacterial host cells. Different strains of *E. coli* are frequently used as recipient cells. Using such plasmid-dependent transformation methods, *E. coli* cells have been engineered to produce a variety of valuable human peptides and proteins, including insulin, γ-interferon, a number of interleukins, superoxidedismutase, plasminogen activator, tumor necrosis factor, erythropoietin, etc. These substances are either already used in medical practice or undergoing different stages of clinical studies.

However, the plasmid method has serious disadvantages. It is technologically complicated, since the desired product has to be extracted from bacterial cells after biosynthesis, which is a multi-stage process. For example, interferon extraction

involves disintegration of cells, buffer extraction, polyethylemenin processing, illumination, sedimentation by ammonium sulfate, dialysis, and centrifugation (Goeddel, EP 0043980). The necessity for such extraction and purification steps not only complicates production technology of the recombinant product, but also results in substantial losses, especially during large-scale industrial production.

A further complicating factor is that at relatively high levels of expression of the cloned genes, the eukaryotic proteins generated tend to accumulate in the cytoplasm of *E. coli* as insoluble aggregates, which are often associated with cell membranes. Consequently, the already difficult extraction and purification methods discussed above should be supplemented with additional technical procedures related to the extraction of the insoluble inclusion bodies. Usually, the insoluble proteins are solubilized using ionic detergents, such as SDS or laurylsarcosine, at increased temperatures or in the presence of denaturants, such as 8 M urea or 6-8 M guanidine-HCl.

Often, the final stage of purification involves renaturation and reoxidation of the solubilized polypeptides, which is required to restore functional activity. Disulfide bonds, which are necessary for proper folding of the protein in its native conformation, should be reformed. Renaturation procedures, such as disulfide interchange, may use expensive and relatively toxic reagents, like glutathione, and oxidized 2-mercaptoethanol or dithiothreitol. Further, the final yield of bioactive genetically-engineered proteins may be relatively low. Moreover, the presence of even trace concentrations of the toxic reagents needed to solubilize and then re-establish secondary and tertiary protein structure may prohibit subsequent clinical application of the proteins. Thus, the generation of targeted protein in the form of insoluble inclusion bodies within the bacterial host cells not only complicates the production of recombinant proteins and results in diminished yield, but may also render the final protein unsuitable for clinical use (Fisher, B., Sumner, I., Goodenough, P. Biotech. and Bioeng. 41:3-13, 1993).

The technological difficulties associated with the extraction of proteins produced by the expression of heterologous genes from plasmid-transformed bacterial host cells may be overcome by infecting the transformed bacterial host cells with bacteriophage, whose lytic pathway results in lysis of the bearer cell. Thus, the desired protein may be

simply released into the culture medium (Breeze A.S. GB 2 143 238A). Accordingly, Breeze disclosed a method of increasing the yield of enzyme produced in *E. coli* by infecting the bacterial cells with phage λ carrying a temperature-sensitive mutation in *c*I to provide controlled lysis. The *c*I-gene product is a repressor of early transcription and consequently blocks transcription of the late region of the phage DNA, which is required for head and tail assembly and cell lysis (Mantiatis, T., Fritsch, E.F., Sambrook, J., MOLECULAR CLONING: A LABORATORY MANUAL, 1982, Cold Spring Harbor Laboratory Press). Bacteriophages carrying a temperature-sensitive mutation in *c*I are able to establish and maintain the lysogenic state as long as the cells are propagated at a temperature that allows the *c*I-gene product to repress transcription of phage genes necessary for lytic growth. Accordingly, the transformed bacterial host cells may be cultivated at 30° C, wherein the *c*I-mediated suppression of phage DNA transcription continues and the phage remains in the lysogenic state, until the stage of maximum ferment production is reached. Subsequently, the culture temperature may be increased to 42° C for 30 minutes in order to inactivate the *c*I repressor and permit the phage to begin its lytic development. The host cells may then be incubated for 2-3 hours at 30° C to allow complete lysis and release of the enzyme (Breeze A.S. GB 2 143 238A).

Although Breeze teaches release of proteins from bacterial producer cells, it requires cultivating producers at temperatures not exceeding 30° C, which is not the optimum temperature for growth of *E. coli* cells. Synthesis at the optimum temperature (37° C) is not possible, since cells at temperatures exceeding 32° C undergo lysis before reaching the stage of maximum ferment accumulation due to the development of temperature-sensitive lytic prophage. Furthermore, incubation of the bacterial host cells at 42° C for 30 min as disclosed by Breeze may activate proteases that destroy the targeted protein.

Auerbach et al. (U.S. Patent No. 4,637,980) used a phage λ DNA fragment for inducing lytic release of recombinant products. In that method, like Breeze, the temperature-sensitive mutation in λ *c*I-gene product was used to provide temperature-dependent lysis of the bacterial host cells. The λ DNA fragment in Auerbach maintained functional endolysin-encoding genes, N, Q, R and S, for producing

lysozyme following inactivation of the *c*I repressor at 42° C. Most of the remaining phage genes were deleted; mutations in O and P genes prevented replication of the phage DNA. Consequently, the λ DNA was not a fully functional phage, capable of modulating expression of the targeted gene. Moreover, the λ DNA of Auerbach was not suitable for use as a vector for carrying targeted genes. Further, as discussed above, incubation of the bacterial host cells at 42° to 44° C for 90-120 min as disclosed by Auerbach may activate proteases that destroy the targeted protein.

In addition to providing for the lytic release of intact protein from bacterial producer cells, bacteriophages have also been used as an alternative to bacterial plasmid-based vectors, for carrying heterologous DNA into host bacterial cells. (Murray, N.E. and Murray, K., *Nature* 251:476-481, 1974; Moir, A., Brammar, W.J., *Molec. gen. Genet.* 149:87-99, 1976). Typically, amplification of genes and their products is achieved during lytic growth of the phage, wherein the phage genome is integrated into the bacterial host DNA (Panasenko, S.M., Cameron, J.R., Davis, R.V., Lehman, L.R., *Science* 196:188-189, 1977; Murray, N.E. and Kelley, W.S., *Molec. Gen. Genet.* 175:77-87, 1979; Walter, F., Siegel, M., Malke, H., 1990, DD 276,694; Mory, Y., Revel, M., Chen, L., Sheldon, I.F., Yuti-Chernajovsky, 1983, GB 2,103,222A). Usually, either lysogenic cultures of recombinant phage λ are used for infecting the bacterial host cells, or "warmed up" bacterial cultures, already harboring recombinant lysogenic phage λ, are grown up to amplify expression of the heterologous genes.

Although there are examples of the successful use of λ vectors for expression of heterologous genes, λ vectors have been used primarily for gene cloning. Once cloned, the genes are transferred to plasmid vectors for more effective expression. For example, when *E. coli* is infected by phage λ Charon 4A C15, containing the human ß-interferon gene, the quantity of interferon in cell lysate constituted 7-8 x 10^6 units/liter. After the DNA fragment bearing targeted gene was recloned from phage to plasmid, ß-interferon yield increased to 1 x 10^8 units/liter (Moir, A., Brammar, W.J., *Molec. gen. Genet.* 149:87-99, 1976).

To increase the yield of heterologous protein generated in bacterial host cells by recombinant λ vectors, mutations in the phage genome have been introduced that cause phage λ to lose its ability to initiate bacterial cell lysis. Enhanced yield is thereby

achieved by extending the period of time during which the heterologous gene is expressed by the bacterial host cells. Thus, for example, the yield of DNA ligase 1 in lysogenic cultures containing λ gt4ligS prophage, with amber-mutation in the S gene, was five times greater than the yield of DNA ligase 1 in lysogenic cultures containing λ gt4lig prophage without the amber-mutation (Panasenko, S.M., Cameron, J.R., Davis, R.V., Lehman, L.R., *Science* 196:188-189, 1977). The phage λ S protein is required for lysis; therefore S^- mutants accumulate large numbers of intracellular progeny phage particles, as well as the targeted protein, without lysing the host cells (Mantiatis, T., Fritsch, E.F., Sambrook, J., MOLECULAR CLONING: A LABORATORY MANUAL, 1982, Cold Spring Harbor Laboratory Press).

Similar increases in the yield of DNA polymerase 1 were reported for lysogenic cultures containing recombinant phage λ with amber-mutations in the S and Q genes, compared to recombinant phage λ without the amber-mutations (Murray, N.E. and Kelley, W.S., *Molec. gen. Genet.* 175:77-87, 1979). The phage λ Q protein is required for transcription of the late region of the phage DNA, which includes many genes involved in head and tail assembly and cell lysis. (Mantiatis, T., Fritsch, E.F., Sambrook, J., MOLECULAR CLONING: A LABORATORY MANUAL, 1982, Cold Spring Harbor Laboratory Press).

In U.S. Patent No. 4,710,463, Murray discloses lysogenizing a non-suppressing (Su°) strain of *E. coli* with phage λ containing the temperature-sensitive mutation in *c*I, as well as mutations in λ S and E genes. Consequently, prolonged cultivation of the lysogenic *E. coli* at 37° C leads to high levels of production of the recombinant protein, which is retained within the cells, since these are not lysed by phage gene products in the normal way, and since the recombinant phage genome is not encapisdated, it remains available for transcription.

Despite the enhanced yield of heterologous proteins possible using λ-vectors with N, R, S, Q and/or E mutations, the potential technical advantages of bacteriophage vectors related to the lytic release of targeted proteins, may be lost with these mutations, because the targeted proteins accumulate inside the bacterial cell. Thus, when a lysis-defective mutant λ-vector is used for production of heterologous protein, the extraction

and purification steps, discussed above for bacterial cells transformed by plasmid vectors, along with the resultant losses, should be performed.

The T7 promoter/T7 RNA polymerase system is useful for high level expression of recombinant proteins. The use of the T7 promoter requires the presence of T7 RNA polymerase. The T7 RNA polymerase may be supplied by induction of a recombinant T7 polymerase gene contained on a λ lysogen in the host strain or by transformation with a plasmid for expression of the T7 polymerase gene. The T7 RNA polymerase is very specific for its own promoter. Transcription reactions from the T7 promoter are very efficient and many copies of full length RNA can be produced from each template.

Summary of the Invention

In one embodiment, a method for producing a biologically active protein is disclosed, including the steps of:

transforming a strain of *E. coli* with a plasmid having at least one copy of an expressible gene encoding a biologically active protein, operably linked to a T7 polymerase promoter, wherein the *E. coli* strain is capable of expressing the gene for T7 RNA polymerase;

infecting the transformed bacterial host cell with a bacteriophage λ capable of mediating delayed lysis; and

cultivating the *E. coli* host cell under a culture condition that induces lytic growth of said cell without lysis until a desired level of production of said protein is reached, wherein said protein is produced as a soluble, biologically-active protein.

In a preferred embodiment, the bacteriophage λ has a temperature-sensitive mutation. In a more preferred embodiment, the temperature-sensitive mutation is cI_{857}. Preferably, the *E. coli* host cells are grown at a temperature which prevents lytic growth of the bacteriophage λ, prior to the cultivating step.

In a preferred embodiment, the bacteriophage λ has a mutation in at least one gene capable of mediating delayed lysis. In a more preferred embodiment, the at least one gene capable of mediating delayed lysis is selected from the group consisting of N, Q and R.

In a preferred embodiment, the strain of *E. coli* produces a suppressor for the repair of amber-mutations.

In a alternate embodiment, the strain of *E. coli* lacks a suppressor for the repair of amber-mutations.

In a preferred embodiment, the infecting bacteriophage λ is provided at a multiplicity of infection in a range of about 1 to about 100. In a more preferred embodiment, the infecting bacteriophage λ is provided at a multiplicity of infection in a range of about 10 to about 25.

Preferably, the bacteriophage-mediated delayed lysis of the strain of *E. coli* is delayed at higher multiplicities of infection relative to lower multiplicities of infection.

In one embodiment, the expressible gene encodes a human acidic fibroblast growth factor. In one alternate embodiment, the human acidic fibroblast growth factor contains 134 amino acids. In another alternate embodiment, the human acidic fibroblast growth factor contains 140 amino acids. In another alternate embodiment, the human acidic fibroblast growth factor contains 146 amino acids. In another alternate embodiment, the human acidic fibroblast growth factor contains 155 amino acids. In a most preferred embodiment, the human acidic fibroblast growth factor has the sequence as set forth in SEQ ID NO: 1.

In one embodiment, the expressible gene encodes a human growth hormone. In an alternate embodiment, the expressible gene encodes a human interferon. In yet another embodiment, the expressible gene encodes an *E. coli* methionine amino peptidase.

In a preferred embodiment, the gene for T7 RNA polymerase is under the control of an inducible promoter. In a more preferred embodiment, the inducible promoter is a lac UV 5 promoter.

In a preferred embodiment, a method of producing a biologically active protein is provided which includes the steps of:

a) growing a first strain of *E. coli* cells, which harbor a strain of bacteriophage λ, wherein the bacteriophage λ has a temperature-sensitive mutation,

b) adjusting the temperature to provide for lysis of the first strain of *E. coli* cells and release of the bacteriophage λ,

c) providing a second strain of *E. coli* cells which have been transformed with a plasmid having at least one copy of an expressible gene encoding said biologically

active protein, said expressible gene being operably linked to a T7 polymerase promoter under the control of an inducible promoter, wherein the second strain of *E. coli* cells may be induced to express the gene for T7 RNA polymerase by addition of an inducer;

d) infecting the second strain of *E.coli* cells with the bacteriophage λ released from the first strain of *E. coli* cells; and

e) incubating the infected second strain of *E. coli* cells in a culture medium containing the inducer, such that protein is produced and released into the culture medium upon lysis of the second strain of *E. coli* cells, wherein said protein is produced as a soluble, biologically-active protein at a concentration greater than 100 microgram /ml.

Also embodied within the presently disclosed invention is a chemically synthesized nucleic acid consisting essentially of the sequence set forth in SEQ ID NO: 1.

For purposes of summarizing the invention and the advantages achieved over the prior art, certain objects and advantages of the invention have been described above. Of course, it is to be understood that not necessarily all such objects or advantages may be achieved in accordance with any particular embodiment of the invention. Thus, for example, those skilled in the art will recognize that the invention may be embodied or carried out in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objects or advantages as may be taught or suggested herein.

Further aspects, features and advantages of this invention will become apparent from the detailed description of the preferred embodiments which follow.

Brief Description of the Drawings

These and other feature of this invention will now be described with reference to the drawings of preferred embodiments which are intended to illustrate and not to limit the invention.

Figure 1 shows the chemically synthesized nucleotide sequence for human acidic fibroblast growth factor (155 amino acids) (SEQ ID NO: 1) which has been

modified by substitution of naturally occurring codons with codons found in highly expressed *E. coli* proteins and the translated amino acid sequence (SEQ ID NO: 2).

Figure 2 shows the modifications made in the chemically synthesized haFGF 155 codons. FGF fr HUMECGFB is the sequence obtained from GenBank (at NCBI) (SEQ ID NO: 3). HaFGF 155 is the chemically synthesized sequence in accordance with one embodiment of the present invention (SEQ ID NO: 1).

Figure 3 shows the pET24-155@rev construct which contains the chemically synthesized haFGF 155 gene (SEQ ID NO: 1).

Figure 4 shows HPLC purified haFGF 155. In the electrophoregram: lane 1, 10 µl of the conditioned medium containing recombinant haFGF 155; lane 2, 7 µl of Heparin-Sepharose purified recombinant haFGF 155 (0.45 µg/µl); lane 3, 14 µl out of 80 µl of HPLC-purified haFGF 155. The unmarked lane at the far left contains molecular weight standards.

Figure 5 shows the pET24-134@rev construct which contains the chemically synthesized haFGF 134 gene (SEQ ID NO: 4).

Figure 6 shows the chemically synthesized nucleotide sequence for human acidic fibroblast growth factor (134 amino acids) (SEQ ID NO: 4) which has been modified by substitution of naturally occurring codons with codons found in highly expressed *E. coli* proteins and the translated amino acid sequence (SEQ ID NO: 5).

Figure 7 shows the pET24-140 @rev construct which contains the chemically synthesized haFGF 140 gene (SEQ ID NO: 6).

Figure 8 shows the chemically synthesized nucleotide sequence for human acidic fibroblast growth factor (140 amino acids) (SEQ ID NO: 6) which has been

modified by substitution of naturally occurring codons with codons found in highly expressed *E. coli* proteins and the translated amino acid sequence (SEQ ID NO: 7).

Figure 9 shows the pET24ap-inf @rev construct which contains the chemically synthesized interferon α-2b gene (SEQ ID NO: 10).

Figure 10 shows the chemically synthesized nucleotide sequence for human interferon α-2b (SEQ ID NO: 10) which has been modified by substitution of naturally occurring codons with codons found in highly expressed *E. coli* proteins and the translated amino acid sequence (SEQ ID NO: 11).

Figure 11 shows a 12.5% SDS polyacrylamide gel containing proteins produced by the phage-dependent method described herein: lane 1, molecular weight standards, 2 μg each standard; lane 2, 40 μl of culture media containing the recombinant FGF 134 protein; lane 3, 40 μl of culture media containing the recombinant FGF 140 protein; lane 4, 40 μl of culture media containing recombinant interferon α2B; lane 5, 40 μl of culture media containing recombinant FGF 155 protein; lane 6, 40 μl of culture media containing recombinant human growth hormone; lane 7, 40 μl of culture media containing recombinant methionine aminopeptidase; lane 8, 40 μl of culture media containing β-galactosidase of *E. coli*.

Figure 12 shows a 12.5% SDS polyacrylamide gel containing recombinant proteins purified according to the presently claimed invention: lane 1, molecular weight standards; lane 2, 5 μg of purified FGF 134 protein; lane 3, 5 μg of purified FGF 140 protein; lane 4, 5 μg of purified FGF 146 protein; lane 5, 5 μg of purified interferon α2B protein; lane 6, 5 μg of purified FGF 155 protein; lane 7, 5 μg of purified methionine amino peptidase protein; lane 8, molecular weight standards.

Detailed Description of the Preferred Embodiment

While the described embodiment represents the preferred embodiment of the present invention, it is to be understood that modifications will occur to those skilled in

the art without departing from the spirit of the invention. The scope of the invention is therefore to be determined solely by the appended claims.

Bacteriophage λ is useful as a vector because more than 40% of the viral genome is not essential for lytic growth. This area of the λ genome, located in the central region of the λ DNA, between genes J and N, may be replaced by heterologous DNA encoding a desired product. That region is transcribed early during infection.

In order to maximize the expression of a targeted gene, whose synthesis information is recorded in the area of phage's early genes, special conditions for the phage's development should be provided to ensure proper replication. Further, transcription of the early area, containing the targeted gene, should be fostered, while transcription of the later genes, involved in cell lysis, should be decelerated. This slows down maturation of the λ particles and subsequent cell lysis. Consequently, the early phage products, including the targeted gene product, will accumulate in the bacterial cells. Deceleration of late transcription, thereby extending expression of the targeted gene, may be accomplished by: (1) mutations of phage genome that block expression of the later genes (2) increased multiplicity of infection, and/or (3) cultivation of the infected bacterial cells at a reduced temperature.

An advantage of infecting producer cells with a bacteriophage is that the phage causes a profound rearrangement of all macromolecular synthesis in the bacterial host cells. By turning off transcription of bacterial genes, phages may increase the copying of the targeted gene, and consequently, increase the output of desired product.

In one embodiment of the present super-production system, phage λ with amber-mutations that delay bacterial lysis (e.g., Q^- and R^- mutations) are provided in a strain of *E. coli*, designated Su°, which lacks the suppressor responsible for correcting amber-mutations in phage λ. In order to obtain a non-suppressing (Su°) strain of *E. coli*, Su° clones are selected from the wild-type Su^+ population. Preferably, a selection marker is inserted into the phage DNA, e.g., tetracycline or ampicillin resistance.

Selection of non-suppressing (Su°) strains of *E. coli*, for example, *E. coli* K 802 was carried out with phage λ cI_{857} Nam7Nam53 bla tet (hereinafter λ bla N'). Strain *E. coli* C600 (λ bla N') served as source of the phage. This phage was obtained by insertion of plasmid pCV 11 (bla tet) at EcoRI site into single-site (EcoRI) vector

carrying ts-mutation in repressor gene (cI_{857}). Then two amber-mutations were introduced into the phage N gene by recombination *in vivo*.

Clones were tested for non-lysogenicity with phage λ clear. In addition to phage λ bla N', phage λ $cI_{857}\,Q_{am117}\,R_{am54}$ was used to check for suppressor.

As is known, phage λ N' mutant is not able to lyse the host cells and is present in cells in the form of extremely unstable plasmids. If the host cells contain suppressor, the amber-mutation is phenotypically corrected, the N protein is synthesized and the phage can develop lytically. This difference in the viability of Su^+ and Su° cells, infected by λ N', is used as a basis for selection of spontaneously appearing Su° revertants from the *E. coli* Su^+ cell population. Phage λ with an inserted plasmid that introduced the ampicillin and tetracycline resistance markers into cells was used to prevent the nonlysing Su° cells from masking the search for mutants. The phage also contains ts-mutation in the repressor gene that permits lytic development of such phage resulting in cell lysis.

If the medium supplemented with ampicillin and tetracycline is inoculated with Su^+ culture after its infection with phage λ bla N' with subsequent growth at 43° C, single suppressor-free cells containing phage λ bla N' in the form of plasmids should develop on plates. Curing the cells from the phage, we should obtain Su° derivatives of the parent cultures. The method can be subdivided into several stages.

1. Infection of Culture With Phage λ bla N'

The culture *E. coli* Su^+ was grown on the M9 medium with maltose at 37° C under intense agitation to a density of 1-2 x 10^8 cells/ml. The cells were infected with phage λ bla N' at a multiplicity of 5-10 particles per cell and incubated for 20 min at 20° C. Under given conditions, the infection efficiency is about 100%, in addition to the bulk of Su^+ cells, the phage also infects single Su° cells.

2. Selection of Suppressor-Free Cells Containing Marker Phage

After infection, cells were plated out on agar medium supplemented with 12 γ/ml tetracycline and 20 γ/ml ampicillin and grown at 43° C. In 24 h, single colonies developed, which were replated on agar medium with antibiotics and grown at 37° C.

3. Curing of the Selected Clones From Phage λ bla N'

Since phage λ N' in the *E. coli* Su° cells is in the form of extremely unstable plasmids, in order to cure from the phage the selected clones were plated on selective agar medium without antibiotics and grown at 37° C. The number of cells that had lost the phage in the first passage on the medium without antibiotics amounted to 12-35%. The selection of such cells was carried out by monitoring the loss of antibiotic resistance and the acquisition of sensitivity to phage λ clear.

4. Testing of Cells for Repressor

The ability of phage λ with amber-mutations to form plaques on lawns of cured clones was checked. Isogenic suppressor-free derivatives of the parent *E. coli* Su^+ strains are clones, on which phage λ bla N' did not form plaques, phage λ cI_{857} Q_{am117} R_{am54} produced 1-3 x 10^5 PFU/ml, and phage λ cI_{857} without mutations in genes Q and R produced 1 x 10^{10} PFU/ml.

Using this method, we obtained Su° revertants of *E. coli* K 802 Su^+. Based on the cell number at the moment of infection and the number of Su° revertants among them, the frequency of occurrence of suppressor-free cells was 3 x 10^{-7}.

In a preferred embodiment, the gene of interest is cloned into pET-24a(+) under the control of the T7 promoter. Any gene of interest may be used in the practice of the claimed invention. Particular examples include but are not limited to human growth hormone, interferon, methionine amino peptidase, human aFGF 134 amino acid form, human aFGF 140 amino acid form, human aFGF 146 amino acid form, and human aFGF 155 form. In an alternate embodiment, the gene of interest may be cloned into both a bacterial plasmid and the λ phage under the control of appropriate promoters. In a most preferred embodiment, chemically synthesized haFGF 155 gene (SEQ ID NO: 1) is cloned into pET-24a(+) under the control of the T7 promoter. The T7 promoter is recognized only by T7 RNA polymerase and is not recognized by the RNA polymerase of *E.coli*. The obtained plasmid with haFGF 155 gene (phaFGF 155) was transformed into *E. coli* BL21(DE3). This strain contains the T7 RNA polymerase gene. The T7 RNA polymerase gene is under the control of the inducible lac UV5 promoter in order to induce T7 RNA polymerase synthesis only when necessary as this protein is toxic for the *E. coli* cell. The induction of the lac promoter is carried out by adding IPTG to the nutrient medium. In order to obtain the haFGF 155 protein, the producer strain,

containing the recombinant plasmid with the haFGF 155 gene, is cultured under conditions of intensive aeration to a cell density of 5 x 10^7 – 5 x 10^9 cells in 1 ml at a temperature of 20-40°C. Then it is infected by lambda phage with the ts-mutation *c*I repressor gene with a multiplicity from 0.1 to 100 phage bodies per cell and incubation is continued at 20-37°C for 2-14 hours. Simultaneously with the phage, IPTG at a concentration of 1 mM is introduced.

The haFGF155 gene encodes a protein containing 155 amino acid residues. However, it has only been possible to isolate two shorter aFGF forms from tissue samples. The two isolated forms contain 140 and 134 amino acid residues. The aFGF form containing 140 amino acids is considered complete, while the aFGF form containing 134 amino acids is considered to be truncated. It has not been possible to extract the aFGF form containing 155 amino acids from tissue samples. It is not known whether the shorter isoforms occur as a normal function of cell processing or as an artefact produced during the isolation procedure by specific proteases in the process of aFGF extraction. Western Blot analysis of the protein produced from the isolated DNA recombinant molecules for the three aFGF forms showed high expression of the 140 and 134 forms and a low expression level of the 155 form.

In a preferred embodiment of the present invention, the gene for human acidic fibroblast growth factor encodes the 155 amino acid form of the aFGF protein and is chemically synthesized (SEQ ID NO: 1). The nucleotide sequence of the haFGF 155 gene has been deduced on the basis of the previously described haFGF 155 amino acid sequence (SEQ ID NO: 2). The amino acid sequence of the synthesized haFGF155 gene does not differ from those previously described such as the translated sequence of the FGF nucleotide sequence of SEQ ID NO: 3. However, the preferred nucleotide sequence of haFGF gene differs from those previously described. In a preferred embodiment of the present invention, the haFGF 155 gene has been chemically synthesized using the codons which are most often used by *E. coli* for intensively synthesized bacterial proteins. Codon usage tables for E. coli are well known and available. See, for example, http://psyche.uthct.edu/shaun/Sblack/codonuse.html. . Chemical synthesis of human aFGF genes was carried out by well known methods (Edge et al. (1983) Nucleic Acids Research 11 (18): 6419-6435).

Alternatively, any gene of interest may be used in the practice of the present invention including, but not limited to, isolated DNA from animal tissues encoding other forms of the haFGF protein known to those skilled in the art including the 146, the 140 and 134 isoforms and any variants, derivatives, analogs or fragments thererof. Also exemplified herein are genes encoding human growth hormone, human interferon and *E. coli* methionine amino peptidase.

Figure 1 shows the complete nucleotide sequence of the haFGF 155 gene, as synthesized by the present inventors (SEQ ID NO: 1) and also a sequence for human acidic fibroblast growth factor from GenBank (SEQ ID NO:3). These two sequences are compared in Figure 2. There are distinctions in 80 codons.

Expression and cloning vectors typically contain a promoter that is recognized by the host organism and is operably linked to the gene of interest. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within 100-1000 base pairs) that control the transcription and translation of particular nucleic acid sequences to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by prokaryotic host cells are known. One skilled in the art would know how to ligate them to a gene of interest using suitable linkers or adaptors to provide appropriate restriction sites.

A preferred promoter system is the *E. coli* bacteriophage T7 promoter system. The *E. coli* bacteriophage T7 promoter is very specific and requires the presence of the T7 RNA polymerase. The T7 RNA polymerase may be supplied by transformation with a plasmid expressing the gene for T7 RNA polymerase or may be supplied by induction of a T7 polymerase gene contained on a λ lysogen in a host strain. The T7 promoter and T7 RNA polymerase are commercially available.

Transformation means introducing DNA into an organism so that the DNA is capable of replication, either as an extrachromosomal element or by integration into the

chromosome. Transformation of prokaryotic cells is performed using techniques well known to those skilled in the art such as treatment with $CaCl_2$ or electroporation.

Super production of the recombinant proteins was achieved by cultivation of the producer strain under conditions which slow down the lytic development of the lambda phage Such conditions include lowered temperature of cultivation and use of amber mutations in late lambda phage genes such as Q and R genes.

The recombinant proteins are accumulated in the culture medium as a soluble protein as a result of the producer strain cells lysis by lambda phage. The output of recombinant protein generally constituted 20% of the soluble proteins accumulated in the culture medium. Debris was removed from the culture medium by centrifugation. The recombinant proteins can then be purified from contaminant soluble proteins and polypeptides with purification procedures, which are well known to those skilled in the art. Such procedures include, but are not limited to, fractionation on an ion-exchange column, ethanol precipitation, reverse phase HPLC, immunoaffinity, SDS-PAGE, ammonium sulfate precipitation, and gel filtration. In the case of haFGF proteins, the haFGF protein was applied to heparin sepharose in order to obtain purified haFGF.

A more detailed description of the present invention is provided below. While the described embodiment represents the preferred embodiment of the present invention, it is to be understood that modifications will occur to those skilled in the art without departing from the spirit of the invention. The scope of the invention is therefore to be determined solely by the appended claims.

EXAMPLE 1

Production of human aFGF 155 by phage-dependent method

Cultures of *Escherichia coli* BL21(DE3) (NOVAGEN) were transformed by plasmid pET24-155@rev (Figure 3), which contains one copy of the haFGF 155 gene encoding human acidic fibroblast growth factor (155 amino acids). Cultures of BL21(DE3) contain a single copy of the gene for T7 RNA polymerase under the control of the inducible lac UV5 promoter in the bacterial genome (Studier et al. (1986) J. Mol. Biol. 189: 113-130). Into the plasmid pET-24a(+) (NOVAGEN) was inserted the chemically synthesized haFGF 155 gene (SEQ ID NO: 1) under the control of the T7 promoter to produce plasmid pET24-155 @rev. Expression of the haFGF 155 gene

begins only after the appearance of T7 polymerase in the cells which is mediated through the induction of the lac UV5 promoter by IPTG.

Cultures of E. coli BL21(DE3) with pET24-155 @rev were grown with shaking at 37°C in LB medium, containing 50 μg/ml kanamycin, to a density of 2 x 10^8 cells/ml. Then the cells were infected with phage λ cI_{857} Q_{am117} R_{am54} at a multiplicity of about 10 phage bodies per 1 bacterial cell and cultivated with shaking at 21°C for about 14 hour. Simultaneously with phage, 1 mM IPTG was introduced into the medium.

Phage λ cI_{857} Q_{am117} R_{am54} was prepared from lysogenic cultures of *E. coli* RLMI, which were grown in LB medium at 30°C with intensive aeration to a density of approximately 1 x 10^8 cells/ml. The lysogenic culture was warmed to 43°C and incubated for 20 minutes to inactivate *c*I repressor. The temperature was then decreased to 37 °C and after 60-70 minutes the bacterial cells underwent lysis, with phages being formed at 1-2 x 10^{10} PFU/ml.

After incubation with the phage-infected cells for 14 hours, debris was removed from the culture medium by centrifugation. The culture medium, containing the haFGF 155 protein was applied to a heparin sepharose column to obtain pure haFGF 155.

The culture medium containing the haFGF 155 was analyzed by SDS-polyacrylamide gel electrophoresis under denaturing conditions and stained with Coomassie Blue. An electrophoregram of the culture medium, containing haFGF 155 protein is compared to purified haFGF protein in Figure 4. Lane 1 shows 10 μl of the culture medium. Lane 2 shows 7 μl of Heparin-Sepharose purified haFGF 155 protein (0.45 μg/μl). Lane 3 shows 14 μl out of 80 μl of HPLC purified ha FGF-155. The unmarked lane at the far left contains molecular weight standards (Amersham Pharmacia Biotech). The production of haFGF 155 protein in phage-infected cultures was about 20% of the total cellular protein. The molecular weight of haFGF 155 was 17, 908 Daltons as determined by densitometer Image Master VDS (data not shown).

Human aFGF 155 produced by the method disclosed above had biological activity based upon the chick membrane assay (Example 6). In addition, purified human aFGF155 showed bioactivity in a cell-based proliferation assay utilizing BALB/c 3T3 fibroblasts (Linemeyer, US Patent No 5401832). The half-maximal stimulation of cell proliferation occurred at a concentration of 32 pg/ml aFGF155.

Unpurified human aFGF155, contained in the bacterial culture medium, also displayed biological activity in the 3T3 fibroblast assay which was equivalent to purified aFGF155, indicating that aFGF155 was synthesized initially in bacteria as a soluble, biologically-active protein.

EXAMPLE 2

Production of Human aFGF 134 amino acid form by phage-dependent method

Cultures of *Escherichia coli* BL21(DE3) (NOVAGEN) were transformed by plasmid pET24-134@rev (Figure 5), which contains one copy of the chemically synthesized gene encoding human aFGF (134 amino acids) (Figure 6; SEQ ID NO: 4). The translated amino acid sequence is shown in SEQ ID NO: 5. Cultures of BL21(DE3) contain a single copy of the gene for T7 RNA polymerase under the control of the inducible lac UV5 promoter in the bacterial genome (Studier et al. (1986) J. Mol. Biol. 189: 113-130). Into the plasmid pET-24a(+) (NOVAGEN) was inserted the human aFGF 134 amino acid form gene under the control of the T7 promoter. Expression of the human aFGF 134 amino acid form gene begins only after the appearance of T7 polymerase in the cells which is mediated through the induction of the lac UV5 promoter by IPTG.

Cultures of E. coli BL21(DE3) with plasmid pET24-134@rev were grown with shaking at 37°C in LB medium, containing 50 μg/ml kanamycin, to a density of 2 x 10^8 cells/ml. Then the cells were infected with phage λ cI_{857} Q_{am117} R_{am54} at a multiplicity of about 10 phage bodies per 1 bacterial cell and cultivated with shaking at 21°C for about 14 hour. Simultaneously with phage, 1 mM IPTG was introduced into the medium.

Phage λ cI_{857} Q_{am117} R_{am54} was prepared from lysogenic cultures of *E. coli* RLMI, which were grown in LB medium at 30°C with intensive aeration to a density of approximately 1 x 10^8 cells/ml. The lysogenic culture was warmed to 43°C and incubated for 20 minutes to inactivate *c*I repressor. The temperature was then decreased to 37 °C and after 60-70 minutes the bacterial cells underwent lysis, with phages being formed at 1-2 x 10^{10} PFU/ml.

After incubation with the phage-infected cells for 14 hours, debris was removed from the culture medium by centrifugation. The culture medium, containing the haFGF

134 protein was applied to a heparin sepharose column to obtain pure human aFGF 134 protein.

EXAMPLE 3

Production of Human aFGF 140 amino acid form by phage-dependent method

Cultures of *Escherichia coli* BL21(DE3) (NOVAGEN) were transformed by plasmid pET24-140@rev (Figure 7), which contains one copy of the chemically synthesized gene encoding human aFGF (Figure 8; 140 amino acids) (SEQ ID NO: 6). The corresponding protein is shown as SEQ ID NO: 7. Cultures of BL21(DE3) contain a single copy of the gene for T7 RNA polymerase under the control of the inducible lac UV5 promoter in the bacterial genome (Studier et al. (1986) J. Mol. Biol. 189: 113-130). Into the plasmid pET-24a(+) (NOVAGEN) was inserted the human aFGF 140 amino acid form gene under the control of the T7 promoter. Expression of the human aFGF 140 amino acid form gene begins only after the appearance of T7 polymerase in the cells which is mediated through the induction of the lac UV5 promoter by IPTG.

Cultures of E. coli BL21(DE3) with pET24-140@rev were grown with shaking at 37°C in LB medium, containing 50 μg/ml kanamycin, to a density of 2 x 10^8 cells/ml. Then the cells were infected with phage λ cI_{857} Q_{am117} R_{am54} at a multiplicity of about 10 phage bodies per 1 bacterial cell and cultivated with shaking at 21°C for about 14 hour. Simultaneously with phage, 1 mM IPTG was introduced into the medium.

Phage λ cI_{857} Q_{am117} R_{am54} was prepared from lysogenic cultures of *E. coli* RLMI, which were grown in LB medium at 30°C with intensive aeration to a density of approximately 1 x 10^8 cells/ml. The lysogenic culture was warmed to 43°C and incubated for 20 minutes to inactivate *c*I repressor. The temperature was then decreased to 37 °C and after 60-70 minutes the bacterial cells underwent lysis, with phages being formed at 1-2 x 10^{10} PFU/ml.

After incubation with the phage-infected cells for 14 hours, debris was removed from the culture medium by centrifugation. The culture medium, containing the haFGF 140 amino acid form was applied to a heparin sepharose column to obtain pure human aFGF 140.

Human aFGF 140 produced by the method disclosed above had biological activity based upon the chick membrane assay (Example 6).

EXAMPLE 4

Production of Human aFGF 146 amino acid form by phage-dependent method

Cultures of *Escherichia coli* BL21(DE3) (NOVAGEN) were transformed by plasmid pET24-146@rev, which contains one copy of the chemically synthesized gene encoding human aFGF (146 amino acids) (not shown). Cultures of BL21(DE3) contain a single copy of the gene for T7 RNA polymerase under the control of the inducible lac UV5 promoter in the bacterial genome (Studier et al. (1986) J. Mol. Biol. 189: 113-130). Into the plasmid pET-24a(+) (NOVAGEN) was inserted the human aFGF 146 amino acid form gene under the control of the T7 promoter. Expression of the human aFGF 146 amino acid form gene begins only after the appearance of T7 polymerase in the cells which is mediated through the induction of the lac UV5 promoter by IPTG.

Cultures of E. coli BL21(DE3) with pET24-146@rev were grown with shaking at 37°C in LB medium, containing 50 μg/ml kanamycin, to a density of 2 x 10^8 cells/ml. Then the cells were infected with phage λ cI_{857} Q_{am117} R_{am54} at a multiplicity of about 10 phage bodies per 1 bacterial cell and cultivated with shaking at 21°C for about 14 hour. Simultaneously with phage, 1 mM IPTG was introduced into the medium.

Phage λ cI_{857} Q_{am117} R_{am54} was prepared from lysogenic cultures of *E. coli* RLMI, which were grown in LB medium at 30°C with intensive aeration to a density of approximately 1 x 10^8 cells/ml. The lysogenic culture was warmed to 43°C and incubated for 20 minutes to inactivate *c*I repressor. The temperature was then decreased to 37 °C and after 60-70 minutes the bacterial cells underwent lysis, with phages being formed at 1-2 x 10^{10} PFU/ml.

After incubation with the phage-infected cells for 14 hours, debris was removed from the culture medium by centrifugation. The culture medium, containing the haFGF 146 protein was applied to a heparin sepharose column to obtain pure human aFGF 146.

Human aFGF 146 produced by the method disclosed above had biological activity based upon the chick membrane assay (Example 6).

EXAMPLE 5

Purification of recombinant haFGF proteins

The culture medium containing a haFGF protein is diluted with one volume of 0.04M KH_2PO_4 buffer, pH 7.0, and applied to a heparin-sepharose column equilibrated

with 0.02 M KH_2PO_4, pH 7.0. The flow rate is adjusted to 80 ml/hour. After application of the culture medium containing the haFGF protein, the column is washed with 0.02M KH_2PO_4 buffer, pH 7.0. Next, the column is washed with 0.02 M KH_2PO_4 buffer containing 0.6M NaCl, pH 7.3. Elution is carried out using 0.02 M KH_2PO_4 buffer with 1.5 M NaCl, pH 7.5. All steps are carried out at 4°C.

EXAMPLE 6

A method of studying FGF influence on the formation of new blood vessels in the chicken embryo chorio-allantoic membrane (CAM).

The method of studying angiogenesis on the model of chicken embryos (Thomas et al. (1985) Proc. Natl. Acad. Sci, USA 82: 6409-6413) was adapted to determine the effects of the haFGF 155, 146, and 140 recombinant proteins on angiogenesis compared to pure brain-derived acidic fibroblast growth factor. Pure brain-derived acidic fibroblast growth factor is a potent angiogenic vascular endothelial cell mitogen with sequence homology to interleukin.

The shells of three-day old chicken embryos were sterilized with ethyl alcohol. The shell and under shell cover were removed from the air chamber using forceps and the eggs were covered by the bottom of a plastic 35 mm Petri dish. The embryos were incubated at 37°C for 5-6 days. At the end of this period, the embryos were examined and the eggs with well-developed blood vessels of CAM were selected for experimentation.

Filter paper disks with deposited gel containing FGF were laid on the eggs CAM with the gel towards the blood vessels and incubated in a thermostat at 37°C for another 3 days. The gel was prepared in the following way: the tested quantity of FGF was dissolved in 30 µl of Eagle's medium (solution 1); then in 30 µl of Eagle's medium, 10 µg of heparin was dissolved and 2% of agarose added (solution 2). Then equal volumes of solution 1 and 2 were mixed and the obtained mixture was deposited in aliquots by 60 µl on 12 mm diameter filter paper disks.

On the 4th day, the filter paper disks were removed. Rich cow milk (10% milkfat) was injected under CAM in a quantity of about 1 ml or less. The result was a white background against which the CAM vessels were easily observed.

The results of the experiment were recorded with a video camera in conjunction with a computer. The formation of new CAM vessel under the affect of FGF was evaluated by the following parameters: the nature and direction of vessel growth, their quantity and quality (large, medium, small), the presence or absence of anastomosis, etc. These data were compared with the control samples which had not been exposed to FGF. Chicken embryo blood vessels on the 14th day of development were treated with FGF155 produced by the phage-dependent recombinant method described herein and purified on heparin sepharose as described.

Application of recombinant FGF155 protein demonstrated the formation of new blood vessels. On the fourth day after application of 1 μg of FGF155, vessels were mainly small and showed radial growth. Increasing the amount of FGF155 to 3 μg showed a corresponding increase in the size of the blood vessels. Medium vessels were observed with radial growth. A further increase to 4 μg of FGF155 applied showed development of large, medium and small blood vessels at 4 days after application as compared to control.

EXAMPLE 7

Production of Human Growth Hormone by phage-dependent method

Cultures of *Escherichia coli* BL21(DE3) (NOVAGEN) were transformed by a plasmid which contains one copy of a chemically synthesized gene encoding human growth hormone (SEQ ID NO: 8). The translated amino acid sequence is shown as SEQ ID NO: 9. Cultures of BL21(DE3) contain a single copy of the gene for T7 RNA polymerase under the control of the inducible lac UV5 promoter in the bacterial genome (Studier et al. (1986) J. Mol. Biol. 189: 113-130). Into the plasmid pET-24a(+) (NOVAGEN) was inserted the human growth hormone gene under the control of the T7 promoter. Expression of the human growth hormone gene begins only after the appearance of T7 polymerase in the cells which is mediated through the induction of the lac UV5 promoter by IPTG.

The transformed cultures of E. coli BL21(DE3) were grown with shaking at 37°C in LB medium, containing 50 μg/ml kanamycin, to a density of 2 x 10^8 cells/ml. Then the cells were infected with phage λ cI_{857} Q_{am117} R_{am54} at a multiplicity of about 10

phage bodies per 1 bacterial cell and cultivated with shaking at 21°C for about 14 hour. Simultaneously with phage, 1 mM IPTG was introduced into the medium.

Phage λ cI_{857} Q_{am117} R_{am54} was prepared from lysogenic cultures of *E. coli* RLMI, which were grown in LB medium at 30°C with intensive aeration to a density of approximately 1 x 10^8 cells/ml. The lysogenic culture was warmed to 43°C and incubated for 20 minutes to inactivate *c*I repressor. The temperature was then decreased to 37 °C and after 60-70 minutes the bacterial cells underwent lysis, with phages being formed at 1-2 x 10^{10} PFU/ml.

After incubation with the phage-infected cells for 14 hours, debris was removed from the culture medium by centrifugation. The human growth hormone protein was purified by column chromatography by methods known to those skilled in the art to obtain pure human growth hormone. The purified human growth hormone was biologically active when assayed in a cell-based bioassay utilizing Nb2 lymphoma cells (Gout PW, *Cancer Research* 40:2433-2436, 1980). The concentration of human growth hormone that gave half-maximal stimulation of Nb2 cell proliferation was 125 pg/ml.

EXAMPLE 8

Production of Human Interferon α-2b by phage-dependent method

Cultures of *Escherichia coli* BL21(DE3) (NOVAGEN) were transformed by plasmid pET24ap-inf@rev (Figure 9), which contains one copy of a chemically synthesized gene encoding α-2 human interferon (Figure 10; SEQ ID NO: 10). The translated amino acid sequence is shown as SEQ ID NO: 11.. Cultures of BL21(DE3) contain a single copy of the gene for T7 RNA polymerase under the control of the inducible lac UV5 promoter in the bacterial genome (Studier et al. (1986) J. Mol. Biol. 189: 113-130). Into the plasmid pET-24a(+) (NOVAGEN) was inserted the interferon gene under the control of the T7 promoter. Expression of the interferon gene begins only after the appearance of T7 polymerase in the cells which is mediated through the induction of the lac UV5 promoter by IPTG.

Cultures of E. coli BL21(DE3) with plasmid pET24ap-inf@rev were grown with shaking at 37°C in LB medium, containing 50 μg/ml kanamycin, to a density of 2 x 10^8 cells/ml. Then the cells were infected with phage λ cI_{857} Q_{am117} R_{am54} at a multiplicity of

about 10 phage bodies per 1 bacterial cell and cultivated with shaking at 21°C for about 14 hour. Simultaneously with phage, 1 mM IPTG was introduced into the medium.

Phage λ cI_{857} Q_{am117} R_{am54} was prepared from lysogenic cultures of *E. coli* RLMI, which were grown in LB medium at 30°C with intensive aeration to a density of approximately 1 x 10^8 cells/ml. The lysogenic culture was warmed to 43°C and incubated for 20 minutes to inactivate CI repressor. The temperature was then decreased to 37 °C and after 60-70 minutes the bacterial cells underwent lysis, with phages being formed at 1-2 x 10^{10} PFU/ml.

After incubation with the phage-infected cells for 14 hours, debris was removed from the culture medium by centrifugation. Interferon was purified by column chromatography by methods known to those skilled in the art to obtain pure interferon.

Interferon produced by the disclosed method had biological activity based upon the interferon antiviral assay performed in vesicular stomatitus virus infected bovine kidney cells (Aebersold P, *Methods in Enzymology* 119:579-592, 1986). Interferon alpha 2b had a biological potency of 1.81 x 10^8 International Units (IU) per mg protein in this assay. Interferon alpha 2b contained in the bacterial culture media prior to purification had an equivalent potency to the purified interferon in this antiviral assay, indication that interferon alpha 2b is initially synthesized in bacteria as a soluble, biologically-active protein.

EXAMPLE 9

Production of *E. coli* Methionine Amino Peptidase by phage-dependent method

Cultures of *Escherichia coli* BL21(DE3) (NOVAGEN) were transformed by a plasmid which contains one copy of a chemically synthesized gene encoding *E. coli* methionine amino peptidase. Cultures of BL21(DE3) contain a single copy of the gene for T7 RNA polymerase under the control of the inducible lac UV5 promoter in the bacterial genome (Studier et al. (1986) J. Mol. Biol. 189: 113-130). Into the plasmid pET-24a(+) (NOVAGEN) was inserted the *E. coli* methionine amino peptidase gene under the control of the T7 promoter. Expression of the *E. coli* methionine amino peptidase gene begins only after the appearance of T7 polymerase in the cells which is mediated through the induction of the lac UV5 promoter by IPTG.

The transformed cultures of E. coli BL21(DE3) were grown with shaking at 37°C in LB medium, containing 50 μg/ml kanamycin, to a density of 2 x 10^8 cells/ml. Then the cells were infected with phage λ cI_{857} Q_{am117} R_{am54} at a multiplicity of about 10 phage bodies per 1 bacterial cell and cultivated with shaking at 21°C for about 14 hour. Simultaneously with phage, 1 mM IPTG was introduced into the medium.

Phage λ cI_{857} Q_{am117} R_{am54} was prepared from lysogenic cultures of *E. coli* RLMI, which were grown in LB medium at 30°C with intensive aeration to a density of approximately 1 x 10^8 cells/ml. The lysogenic culture was warmed to 43°C and incubated for 20 minutes to inactivate CI repressor. The temperature was then decreased to 37 °C and after 60-70 minutes the bacterial cells underwent lysis, with phages being formed at 1-2 x 10^{10} PFU/ml.

After incubation with the phage-infected cells for 14 hours, debris was removed from the culture medium by centrifugation. *E. coli* methionine amino peptidase was purified by column chromatography by methods known to those skilled in the art to obtain pure *E. coli* methionine amino peptidase.

EXAMPLE 10

Gel Analysis of recombinant proteins produced by the phage-dependent method.

Culture media containing human aFGF 134 amino acid form, human aFGF 140 amino acid form, human aFGF 155 amino acid form, human growth hormone, interferon, and methionine aminopeptidase were analyzed by SDS-polyacrylamide gel electrophoresis under denaturing conditions and stained with Coomassie Blue. An electrophoregram of culture media, containing human aFGF 134 amino acid form, human aFGF 140 amino acid form, human aFGF 146 amino acid form, human growth hormone, and interferon proteins is compared to molecular weight standards in Figure 11. Lane 2 shows 30 μl of the culture medium containing human aFGF 134 amino acid form. Lane 3 shows 30 μl of culture media containing the recombinant FGF 140 protein. Lane 4 shows 30 μl of culture media containing recombinant interferon. Lane 5 shows 30 μl of culture media containing recombinant FGF 155 protein. Lane 6 shows 30 μl of culture media containing recombinant human growth hormone. Lane 7 shows 30 μl of culture media containing recombinant methionine aminopeptidase. Lane 1 shows 2 μg of each molecular weight standard (Amersham Pharmacia Biotech). From

the top, the molecular weight standards are: 94,000; 67,000; 43,000; 30,000; 20,100; and 14,400.

Quantitation of amounts of human aFGF 134 amino acid form, human aFGF 140 amino acid form, human aFGF 155 amino acid form, human growth hormone, interferon, and methionine aminopeptidase in a mixture was accomplished by scanning the stained protein bands on a polyacrylamide gel with densitometer Image Master VDS (Pharmacia Biotech). The production of the recombinant proteins in phage-infected cultures was about 20% of the total cellular protein.

An electrophoregram containing purified purified recombinant human aFGF 134, haFGF 140, ha FGF 146, interferon, haFGF 155 and methionine aminopeptidase protein was compared to molecular weight standards (Figure 12). Lane 2 shows 5 μg of the purified aFGF 134 protein. Lane 3 shows 5 μg of the purified human aFGF 140. Lane 4 shows 5 μg of the purified human aFGF 146 amino acid form. The production of human aFGF 146 amino acid form in phage-infected cultures was about 20% of the total cellular protein. Lane 5 shows 5 μg of purified interferon. Lane 6 shows 5 μg of haFGF 155 protein. Lane 7 shows 5 μg of the purified *E. coli* methionine amino peptidase. Lanes 1 and 8 show 2 μg of each molecular weight standard (Amersham Pharmacia Biotech).

It will be understood by those of skill in the art that numerous and various modifications can be made without departing from the spirit of the present invention. Therefore, it should be clearly understood that the forms of the present invention are illustrative only and are not intended to limit the scope of the present invention.